European canine lymphoma network consensus recommendations for reporting flow cytometry in canine hematopoietic neoplasms

Regional spotlight

EUROPEAN CANINE LYMPHOMA NETWORK CONSENSUS RECOMMENDATIONS FOR REPORTING FLOW CYTOMETRY IN CANINE HEMATOPOIETIC NEOPLASMS

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Running title: reporting flow cytometry in canine lymphoma

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Abstract

Background: Flow cytometry (FC) is assuming increasing importance in diagnosis in veterinary oncology. The European Canine Lymphoma Network (ECLN) is an international cooperation of different institutions working on canine lymphoma diagnosis and therapy. The ECLN panel of experts on FC has defined the issue

of reporting FC on canine lymphoma and leukemia as their first hot topic, since a standardized report that includes all the important information is still lacking in veterinary medicine.

Methods: The flow cytometry panel of the ECLN started a consensus initiative using the Delphi approach. Clinicians were considered the main target of FC reports. A panel of experts in FC was interrogated about the important information needed from a report.

Results: Using the feedback from clinicians and subsequent discussion, a list of information to be included in the report was made, with four different levels of recommendation. The final report should include both a quantitative part and a qualitative or descriptive part with interpretation of the salient results. Other items discussed included the necessity of reporting data regarding the quality of samples, use of absolute numbers of positive cells, cutoff values, the intensity of fluorescence, and possible aberrant patterns of antigen expression useful from a clinical point of view.

Conclusion: The consensus initiative is a first step towards standardization of diagnostic approach to canine hematopoietic neoplasms among different institutions and countries. This harmonization will improve communication and patient care and also facilitate the multicenter studies necessary to further our knowledge of canine hematopoietic neoplasms.

Introduction

Flow cytometry (FC) is increasingly being used in veterinary clinical pathology laboratories owing to the increasing number of FC facilities, greater availability of specific antibodies labeled with different fluorochromes, and the rapidity with which results can be generated. Immunophenotyping hematopoietic neoplasms is one of the most important applications of FC in veterinary clinical pathology diagnostics since this method rapidly provides useful information on the lineage of neoplastic cells, identifies some specific neoplastic subtypes (T zone lymphomas, chronic lymphocytic leukemia) (1-4), accurately defines stage (5) and, in some cases, can detect minimal residual disease (6).

The overall utility of flow cytometry and its role in the diagnostic pathway is dependent on several aspects. One aspect is the ability to provide important information to frame the neoplastic disease in the context of results of other clinical and laboratory tests. Therefore, a useful FC report should contain all the appropriate information required by clinicians, provide improved characterization of the neoplastic disease, help determine therapy, and inform a monitoring strategy to enable early detection of relapse. This requires sufficient clarity of reporting to allow understanding by non-FC experts. The specific experience of the flow cytometrist should help in the interpretation of the results, always in conjunction with signalment, clinical presentation, and the results of other laboratory tests. However, an appropriate report should also contain all necessary raw data and describe the strategies used to generate them. This provides information about reproducibility and facilitates interpretation by other experts in second opinion or multi-center studies. The balance between these two aspects, the clinician-friendly utility and the inclusion of sufficient technical information to represent a rigorous and reproducible analysis, shapes the format of the FC report.

In human medicine, the issue of reporting flow cytometry immunophenotyping has been widely debated (7,8) and guidelines have been published and regularly updated (9,10). Until now, there has been no similar discussion in veterinary medicine. To the authors' knowledge, every veterinary FC facility uses its own strategy and system in reporting results of immunophenotyping.

Methods

The European Canine Lymphoma Network (ECLN) is a network that was created in 2009 with the aim of establishing cooperation among different institutions working on canine lymphoma diagnosis and therapy (11). The definition of consensus guidelines and approaches is one of the main goals of ECLN and a specific panel on FC has been formed gathering 16 participants selected on demonstrated expertise in veterinary FC. The ECLN aims to drive the creation of consensus guidelines, possibly interfacing with extra EU specialists, in a democratic and inclusive way. The FC panel of the ECLN defined the issue of reporting FC immunophenotyping as the first "hot" topic requiring a consensus discussion.

When attempting to reach a consensus on defined topics using online-based surveys, a tool called the Delphi method is considered the best technique (12). This method relies on a series of questionnaires provided to the panel participants in 'rounds' until answers converge towards a common answer. Only statements reaching at least 75% consensus agreement are included in the final document, otherwise statements are re-written and additional rounds of questions follow until the threshold agreement level of 75% is reached. After review of the results of the explorative survey and the relevant reference sources, the members of the FC panel are approached through successive rounds of questioning and the answers and feedback are collected in an anonymous fashion. The statements passing the threshold are finally considered as common recommendations and contribute to the consensus paper.

The panel of experts in flow cytometry of the ECLN was also interrogated for each piece of information with 4 different levels of recommendation: mandatory, recommended, additional or irrelevant. Results on the percentage of agreement are reported in Table 1 (16/16 responders). Relevance of information was based on a 75% response threshold. If >75% of respondents answered “mandatory”, “recommended” or “additional” for a specific category without either response necessarily reaching 75% individually, the category was considered “relevant”. The consensus level of recommendation was reported if it reached at least 50% of agreement among responders. If agreement was less than 50% for any level, the final recommendation was reported as “no consensus”.

Before starting with the discussion on different issues, the panel of experts in FC decided to document the perceived needs of clinicians. A preliminary exploratory survey among the members of the ECLN therapy working group, consisting of clinicians interested in the study and therapy of canine lymphoma, was conducted. The complete results obtained from this survey are not included in detail in the present document but were used as a starting draft for the members of the FC panel for discussion in order to define the relevance of each component to be submitted to this consensus evaluation

Results

Preliminary exploratory survey among the members of the ECLN therapy working group

Thirty out of 66 clinicians (45%) of the ECLN therapy working group responded to the preliminary survey. Most (51.7%) respondents reported requesting FC in more than 80% of lymphoma cases, and 17.2% of respondents reported requesting FC in more than 50% but less than 80% of lymphoma cases. Only 24.1% of respondents reported the use of FC in less than 20% of lymphoma cases. Most respondents (51.7%) also reported sending blood and bone marrow samples for staging in selected cases, while 63.3% stated that they usually add smears from blood, bone marrow or lymph node for concurrent cytomorphologic evaluation.

The majority of clinicians (58.6%) reported the definition of the immunophenotype of the neoplasm as the main reason for requiring FC, while refining lymphoma subtype (20.7%), definition of prognosis (6.9%), checking minimal residual disease (3.4%) and differentiating lymphoma from reactive conditions (3.4%) were reasons reported less frequently.

The minimally invasive nature of sampling (40%), the accuracy in resolving reactive vs. neoplastic conditions (16%) and the rapidity of the results (12%) were recognized as the main advantages of FC over other techniques. The most important mentioned characteristics required for a flow cytometric report were that they should first be both exhaustive and accurate (46.4%) and second, easy to read and interpret (42.9%).

Results were variable among respondents, indicating that the needs of clinicians are quite heterogeneous. However, the following components were considered to be essential information, with a consensus of more than 75% of responders: specification of the type of tissue sent, assessment of the quality of the sample, reliability of documented marker expression dependent on sample quality, panel of antibodies applied and aberrant patterns identified. All the other information (the same detailed in Table 1) was generally considered as important or essential (although without 75% consensus), except specification of the instrument used for analysis, which was considered irrelevant information by most clinicians.

Identification of discussion topics

Two major and eight minor issues were identified and subsequently discussed, the discussion including published sources both in human and veterinary medicine.

1.Major issues

a. Identification of the "target users and/or recipients" of FC reports and their needs

The identification of the final "targets" of FC immunophenotyping reporting on canine lymphomas and leukemias is crucial for appropriately defining the relevant information to be included in the report.

Three main "target user and/or recipient groups" with different needs were identified: 1) owners, interested in understanding their pet's disease; 2) clinicians, mainly specialists in oncology, interested in characterizing the disease in order to define prognosis, target therapy and monitor follow-up and 3) clinical pathologists, sometimes specialists in FC, asked to interpret the results of laboratory tests in an integrated way or to provide a second opinion. The relevance and emphasis of each piece of information provided in the FC report is variable among these three user groups.

Clinicians were considered the most important target users of FC reports as they decide if the FC analysis is indicated, provide the samples, communicate the results to owners and institute treatment.

Pet owners were not considered the major target of FC reports since management by the clinician/oncologist is the most common situation. They are generally interested in an accurate diagnosis of the disease from which their pet is suffering. Bibliographic references to support the diagnosis and better clarify the disease biology should be considered optional items in generating a final report. Mentioning the operator/specialist's name and titles should be included as well.

Finally, other specialists such as clinical pathologists and flow cytometrists were considered possible targets of FC reports. Their needs are generally much more related to technical aspects, such as data on gating strategies, possible artefactual changes, viability/conservation of the sample, raw percentages (and absolute numbers) of neoplastic cells, type of labeling used (multicolor vs monocolor; composition of tubes, antibody clones used), and controls. Possibly scattergrams could be used to better clarify some of the technical aspects. However, the inclusion of such technical information in the standard FC report could appear extraneous and lead to confusion of non-experts in FC. This information should be omitted from the standard FC report but could remain available to be provided upon request, including, if necessary, raw FCS files.

b. reporting percentages vs descriptive report

Percentages and possibly absolute numbers commonly form the basis for FC immunophenotyping. This method is considered more objective and accurate; however, a series of problems may be encountered: 1) percentages are directly dependent upon gating strategy (scatter properties vs CD45 positive cells vs specific subtypes) 2) percentages of positive events do not provide any information regarding co-expression 3) percentage of positivity is highly dependent on controls used to set cutoff values 4) providing percentage of positive events alone often cannot distinguish neoplastic and residual normal cells and 5) percentages of positive events may be redundant and may not contribute to clarity and easy interpretation of the results. Reporting percentages of positive events in different cell subpopulations, identified upon light scatter or immunophenotypic features (for instance high FSC low SSC cells, or CD21 positive cells) may further improve accuracy of the report.

In contrast, a descriptive report may better focus on neoplastic cells, is clearer and easier to interpret, avoids redundancies and may provide information on co-expression, aberrant pattern(s) and quantitative expression. However, it may be less objective since it is often biased by the interpretation and experience of the specialist. It is recommended that objective (percentage of positive cells) and subjective (i.e. descriptive interpretation based on experience) statements be clearly identifiable in the report. The descriptive report should be conversational and may easily include data from other laboratory tests (cytological review, molecular clonality assessment, CBC, etc).

Consensus was reached regarding the necessity to include both parts in the FC report, with an emphasis on the conversational, descriptive part. Data such as the percentages of positive cells should be reported in parentheses or in a table attached to the written report. Data about cells not considered important for the tumor subtype (for instance myeloid cells in lymphomas, T cell subsets in B cell neoplasms, residual lymphoid population in AML) should be reported, but with an effort to clearly identify them as additional information (in the conversional part) in order to avoid confusion and redundancies. These data could be discussed in more detail if they have been shown to be related to immunity against the tumor or have possible prognostic meaning based on published research.

2 Minor issues

a. Information regarding the quality of the sample

Information regarding the quality of the sample is crucial for interpreting the results of analysis and identifying any possible sources of bias. Unanimous consensus was reached about the relevance of including information regarding the quality of the sample and the evaluation of viability and preservation of cells in the report, including the type of technique used. This was considered mandatory information by the majority of the participants. These data may be derived from the evaluation of scatterplots, the evaluation of viable cells by using specific stains (propidium iodide, trypan blue, etc) or using other qualitative methods. Objective methods (propidium iodide or other stains) are preferable and results may be reported as percentage of viable cells or with a descriptive method. The technique used to assess the quality of the sample should preferably be specified in the final report.

b. Gating strategies

Although gating strategy used may be considered unnecessary information for clinicians and owners, this piece of information could be of use for specialists in order to better interpret results and for second opinions. Consensus was reached in considering it as relevant information to be possibly included in an FC report but no consensus was reached about the level of recommendation.

c. Dot plot images

Attaching images of dot plots to final reports could be of some use to other specialists to better understand gating strategies. However, they may be of limited use and difficult to interpret for most users (clinicians and pet owners) and may lead to potential misinterpretation. In addition, the choice of plots (histogram vs dot vs tridimensional) is not standardized and the results of all the antibodies used are not easily summarized in a few images. The routine inclusion of dot plots in a final report was not favored. Images or preferably .fcs files should be kept and can be provided upon request.

d. Reporting absolute numbers

Reporting absolute numbers of leukocyte subpopulations may be done by directly counting cells with the flow cytometer or by calculating them from flow cytometric percentages and complete blood count (CBC) data. Reporting absolute numbers could be useful mainly in blood, while they

are probably of limited importance in bone marrow and lymph node aspirates in which relative percentages (out of CD45 positive cells or total cells) are much more important. When absolute numbers are reported, laboratory specific reference intervals should be provided.

e. Cutoff value

The cutoff value for considering a neoplastic population positive or negative for a specific antibody is another important issue. The determination of the percentage of positive neoplastic cells depends on the appropriate negative controls used and may be variable among observers. The use of isotype or fluorescence minus one (FMO) controls is strongly encouraged to correctly define background staining and fluorescence spillover. An internal control (biological comparison control) i.e. a negative population of cells from residual or non-neoplastic cells, is also mandatory to correctly define a cutoff value. However, for the sake of clarity, the results of controls should not be included in the final report. Some authors (13) suggested 20-30% as the lower limit to define a population as positive to a specific antigen but this value has been reconsidered in human medicine. Other authors reported positivity as less than 10% of cells = low, 10-50% intermediate, >50% = high. No consensus was reached about a cutoff percentage value to define a cell population as positive, even though the majority of participants identified 20-30% as an acceptable value. The cutoff of positivity may depend upon the tissue analyzed (lymph node vs peripheral blood vs bone marrow) and the antigen investigated. The issue of controls and cut-off values was considered a crucial issue but beyond the scope of the present consensus paper (reporting flow cytometry results). Owing to its critical importance, this issue will be the focus of a future consensus document of the ECLN. Regarding this paper, consensus was reached about the need to include appropriate controls in the flow cytometric procedure, but inclusion of the control results in the final report is not encouraged.

f. Reporting quantitative antigen expression

This may be useful for common lineage antigens (CD45, CD44, CD18) or activation antigens (Ki67, MHC II) and may provide information about maturation status, aberrant expression or prognosis. In dogs, common lineage antigens have been reported to show different expression intensities in hematopoietic subsets and different maturation and activation stages (14, 15). MHC II has been reported to be associated with prognosis in canine B cell lymphoma (16). Ki67 has been reported to be useful in differentiating low and high grade lymphomas (17). Quantitative aberrancies have been reported in different lymphoma subtypes (18). Reporting intensities of antigen expression may be useful for markers with possible prognostic significance or for those expressed differently than expected.

Quantitative expression is generally reported categorically as *bright* or *dim* but this may be subjective and poorly repeatable. Some authors reported antigen expression as *bright* or *dim* when a difference of at least 15% in fluorescence channels was present in the neoplastic population compared to the residual population of the same lineage (18). This method is repeatable among different laboratories but it is quite complicated and it requires a clear identification of non-neoplastic residual cells of the same phenotype. Other authors (16) compared Mean Fluorescence Index (MFI) of a specific antigen (MHC II) in neoplastic cells to that derived from a cohort of neoplastic cases; expression was reported as “dim” if MFI was lower than the 15^{th} percentile of all MFI calculated from a large series of canine lymphomas. This method is probably easier but it requires a different specific standardization from each laboratory to define the adequate cutoff value for fluorescence and a large caseload to calculate appropriate reference values. In addition, the expression of some antigens could be compared with those of a non-neoplastic reference population clearly identifiable in the sample (such as neutrophils in peripheral blood) (14). This

method is easy to perform and does not require any specific standardization but it is quite subjective and it is based on the assumption that antigen expression remains constant in the reference population. Finally, antigen expression could be accurately quantitated using a curve with calibrated beads but this method is expensive and difficult to apply to clinical/diagnostic conditions. In human medicine, the issue of antigen expression is also crucial. Intensity of staining for some antigens may be expressed as *bright*, *dim* or *negative,* and according to their distribution as *heterogeneous* or *homogeneous*. Some authors also suggest possible reporting of expression as *weak* or *strong*. *Strong* refers to unequivocal positivity (not necessarily to *bright* expression) (8). Other authors suggest defining intensity of some antigens as *low* (when the histogram is significantly different but not easily separable from the negative control), *middle* (when the fluorescence peak is contiguous to the negative control but completely distinguishable from it) or *high* (when the fluorescence peak is two or three logarithmic decades higher than the negative control) (19). Some antigens (such as ZAP-70 protein in human CLL) are reported comparing MFI of neoplastic cells to those of T cells in the same sample. This method is reported as easy to perform and optimal for accurately predicting outcome in CLL (20).

Since no definitive rules on the best way to report fluorescence intensity of antigens in FC reports of canine hematopoietic neoplasms have been generated to date, some recommendations are proposed: 1) antigen expression may be preferentially expressed as *dim* or *bright* and data on distribution of the antigen (homogeneous vs heterogeneous) should be provided only if useful to discriminate between normal and neoplastic populations, to define subtype, to track infiltration/residual disease of neoplastic cells (when quantitative aberrant patterns are present) or to define prognosis; 2) when this quantitation is reported, the definition of neoplastic cells as *dim* or *bright* should be well standardized for each antigen and consistent with methods from published references 3) in the absence of specific published references, the criteria used for defining quantitative antigen expression (*dim* vs *bright*) could be provided in supplementary notes, together with the putative biological meaning 4) when possible, quantitative expression of antigens should be compared with the closest normal hemic population 5) quantitative findings that are irrelevant to clinical importance (i.e. not involving staging, prognosis, minimal residual disease, etc.) should be avoided 6) data regarding in-progress studies should be omitted from the diagnostic report until they complete the peer-review and publication process. If necessary, references of published FC entities could be provided in the notes.

g. Reporting aberrant patterns

Qualitatively and or quantitatively aberrant patterns have been reported in several studies in different lymphoma subtypes in dogs (18,21,22) and may differ from the human counterparts. Their importance is far from completely elucidated but, in some cases, specific aberrant patterns may be useful to define specific subtypes, or to accurately track neoplastic infiltration in tissues and to detect minimal residual disease. In particular, a specific subtype of canine lymphoma with peculiar aberrant patterns different from its human counterpart, T zone lymphoma, is well recognized. This indolent T cell lymphoma subtype is not uncommon in the dog, in contrast to people, and exhibits a characteristic decreased expression of CD45 and frequently the concurrent aberrant expression of CD21 (1,2). The likelihood of detecting an aberrant phenotype is linked to the number of antibodies used and to the type of labeling and analysis performed (mono- vs multi-color). Although evidence of the prognostic role of aberrancies in canine hematopoietic neoplasms is largely still lacking, it is likely that some specific aberrancies could have a correlation with biological behavior, similar to what has been reported in human medicine (23-28). Clinicians are

often interested in the presence of aberrant patterns, although they may tend to overestimate their meaning. The report of percentages of positive cells alone may miss the detection of specific aberrant patterns. The presence of a specific aberrant pattern in neoplastic cells should be indicated in the descriptive part of the report if it may be useful to document the infiltration of neoplastic cells in organs, to monitor therapy and minimal residual disease, to facilitate the early detection of relapse or if it may have a specific biological meaning (prognosis, response to therapy, etc).

Adding a statement to the descriptive part of the report regarding possible FC marker(s) to check infiltration or monitor follow-up and detect relapse may be helpful and should be encouraged. These markers may include single labeling (for instance $CD34^+$ cells in acute leukemias), multiple labeling (e.g. $CD3^+CD45^-$ cells for T zone lymphomas) or fluorescence and morphological aspects together (e.g. large $CD21^+$ cells for DLBCL).

h. Integration of other clinical data

Moving toward an integrated report including hematologic, cytologic, histopathologic, immunohistochemical and molecular biologic data is a major goal. However, the availability of results will influence the possibility of a report integrating all of the laboratory results. An integrated report including hematologic, cytologic and FC results while waiting for results of other ancillary techniques may be a good compromise and is encouraged.

Proposal of a possible template for reporting FC results

According to the previously discussed issues, the proposed report for FC immunophenotyping of canine hematopoietic neoplasms should include several sections.

1) *Laboratory identification*
2) *Patient identification*
3) *Type and quality of the sample(s)*
4) *Sample preparation and staining*
5) *Percentages of positive cells*
6) *Descriptive report*
7) *Diagnosis and interpretation*
8) *Comments and references*
9) *Signatures*

For each section some mandatory, recommended and additional information was identified (Table 1).

Conclusion

The present paper is a first step towards standardization of the flow cytometric approach for canine hematopoietic neoplasms among different institutions and countries. It should help to provide a more accurate report to users and support the use of flow cytometric immunophenotyping in the diagnostic algorithm for canine lymphoma and leukemia. The creation of consensus documents on other important issues, including pre-processing, instrument standardization and maintenance, controls and cut-offs, and suggested antibody panels is an ongoing process for the flow cytometry panel of the ECLN.

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Table 1 legend :

Results of the consensus survey among ECLN flow cytometry panel on recommended information in different sections of a canine hematopoietic neoplasm flow cytometry report. Each piece of information was considered “relevant” if more than 75% of responders classified it as “mandatory”, “recommended” or “additional". Recommendations for each piece of information reflect the category at which > 50% of responders concurred. Failure to achieve these thresholds resulted in a designation of no consensus. Relevancy or recommendation scores of 100% are in bold.

Table 1

		Percentage of agreement (%)				Relevance of information	Recommendation
		mandatory	recommended	additional	irrelevant		
Laboratory identification	Name of the laboratory	100	0	0	0	**Relevant**	**Mandatory**
	Postal address	53	27	13	7	Relevant	Mandatory
	Telephone number	60	40	0	0	**Relevant**	Mandatory
	e-mail contact	60	40	0	0	**Relevant**	Mandatory
	Web page	13	47	27	13	Relevant	No consensus
	Authorization number or licenses	20	40	27	13	Relevant	No consensus
Patient identification	Date of the report	93	7	0	0	**Relevant**	Mandatory
	Date of analysis	64	36	0	0	**Relevant**	Mandatory
	Internal ID code	60	40	0	0	**Relevant**	Mandatory
	Owner's name	80	13	7	0	**Relevant**	Mandatory
	Referring physician name and institution	53	40	7	0	**Relevant**	Mandatory
	Species	93	7	0	0	**Relevant**	Mandatory
	Breed	67	33	0	0	**Relevant**	Mandatory
	Gender	60	40	0	0	**Relevant**	Mandatory
	Age	73	27	0	0	**Relevant**	Mandatory
	Patient name	60	27	7	7	Relevant	Mandatory
	Previous therapy	33	13	54	0	**Relevant**	Additional
	Clinical history	40	20	40	0	**Relevant**	No consensus
	Other laboratory results (CBC, Diff)	27	40	33	0	**Relevant**	No consensus
Type and quality of the sample	Type of tissue(s)	93	7	0	0	**Relevant**	Mandatory
	Type of sample (aspirate, biopsy, blood, fluid, etc)	73	27	0	0	**Relevant**	Mandatory
	Quality of the sample (estimated)	73	27	0	0	**Relevant**	Mandatory
	Sampling data	73	27	0	0	**Relevant**	Mandatory
	Percentage of viable cells	26	60	13	0	**Relevant**	Recommended
	Technique used for viability estimation	13	73	13	0	**Relevant**	Recommended
	Specimen number	33	53	13	0	**Relevant**	Recommended
Cell preparation and staining	Antibodies used	87	13	0	0	**Relevant**	Mandatory
	Cell preparation (whole sample, scraping, Ficoll, RBC lysis)	20	53	27	0	**Relevant**	Recommended

	Antibody clone	0	13	60	27	No consensus	Additional
	Fluorochrome combination	0	13	60	27	No consensus	Additional
	Composition of tubes	0	7	73	20	Relevant	Additional
	Type of controls	0	27	60	13	Relevant	Additional
	Instrumentation	0	13	53	33	No consensus	Additional
	Expected positivities for each antibody	13	33	40	13	Relevant	No consensus
Descriptive report	Qualitative description of immunophenotype of cells of interest	78	14	7	0	**Relevant**	Mandatory
	Comment on quantitative expression of relevant markers	53	27	20	0	**Relevant**	Mandatory
	Aberrant patterns	67	33	0	0	**Relevant**	Mandatory
	Information about staging	7	80	13	0	**Relevant**	Recommended
	Information about residual cells	0	73	27	0	**Relevant**	Recommended
	Qualitatitive description of scatter aspects of cells of interest	47	40	13	0	**Relevant**	No consensus
Percentage of positive cells	Percentage of positive cells in gated population	53	47	0	0	**Relevant**	Mandatory
	Percentage of positive cells in whole population	29	57	7	7	Relevant	Recommended
	Intensity of staining (dim vs bright, homogeneous)	20	60	20	0	**Relevant**	Recommended
	Absolute count on positive cells (in peripheral blood only)	20	53	20	13	Relevant	Recommended
	CD4/CD8 ratio	7	27	53	13	Relevant	Additional
	Representation of histogram/plot	7	20	60	13	Relevant	Additional
	Fluorescence index (quantitative)	0	40	47	13	Relevant	No consensus
	Gating procedure	13	33	33	20	Relevant	No consensus
	Reference intervals for each antigen	7	40	47	7	Relevant	No consensus
Diagnosis and interpretation	Diagnosis of immunophenotype	100	0	0	0	**Relevant**	Mandatory
	Lymphoma subtype (tentative)	53	40	7	0	**Relevant**	Mandatory
	Stage	20	60	7	13	Relevant	Recommended
	Suggested FC markers for monitoring stage and MRD	0	67	33	0	**Relevant**	Recommended

	Possible prognostic factors	27	53	20	0	**Relevant**	Recommended
	Grade (high vs low) (tentative)	40	40	20	0	**Relevant**	No consensus
Comments	Clinical and outcome information	0	64	36	0	**Relevant**	Recommended
	Additional tests suggested	0	53	40	7	Relevant	Recommended
	References	0	47	53	0	**Relevant**	Additional
Signatures	Party responsible for the service	73	20	7	0	**Relevant**	Mandatory
	Flow cytometrist	33	47	20	0	**Relevant**	No consensus